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The goals of this proposal are to evaluate self-reported conditions, determine potential risk factors, and determine if there is a definable immunologic abnormality or evidence of mycoplasma infection.

Of the 1,541 Persian Gulf War Veterans enrolled in the Birmingham registry, 790 chart reviews have been. 718 second physician review has been completed. Forty-eight individuals have an undefined illness with symptoms related to at least three organ systems. There are 72 more patients pending second physician review.

We have categorized the list of veterans who served in the Persian Gulf from the Birmingham catchement area, who will be our in-theater, asymptomatic individuals.

Third, we are identifying individuals in the Birmingham catchement area who will be our disabililty non-theater controls.

Dr. Everson has established the methodology in his laboratory to determine if there are immunological differences in symptomatic Persian Gulf War Veterans and the two control groups.

Dr. Watson has continued to refine the techniques to determine if there has been evidence of mycoplasma exposure in the test and control groups. This has resulted in a manuscript, "Genotyping of *Mycoplasma fermentans* strains based on insertion sites of a mobile genetic element" that has been submitted for publication.

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FOREWORD

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Karen O Blackburn 1/20/98

PI - Signature Date

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5. INTRODUCTION:

Soon after the resolution of the Persian Gulf War, veterans were evaluated for a variety of complaints alleged to be related to the conflict. Many of the complaints were vague and relatively non-specific (i.e. myalgias, arthralgias, headaches or memory loss). However, in association with intense media publicity the term Persian Gulf War syndrome was coined. Attempts to define a specific clinical spectrum seen in this "disorder" have not been successful. Nonetheless, a number of potential causes have been discussed and include exposures to organophosphates, pyridostigmine, petrochemical combustion products, depleted uranium from projectiles and exposure to a variety of endemic infectious agents including leishmania and mycoplasma. Additionally, there are a few isolated reports of identification in the urine of organisms from symptomatic veterans and treatment successes with long-term antibiotics or demonstration of genetic material from mycoplasma in blood of symptomatic veterans. It is not clear from these reports that such studies have been adequately controlled, nor have they been replicated.

There are other reports that certain toxins may be related to alterations in white blood cell cellular functions, particularly in terms of oxidant production, chemotaxis, and other measures of immunological function such as spontaneous immunoglobulin production, delayed type hypersensitivity, proliferative responses to certain mitogens and antigens, and even circulating levels of markers such as the soluble IL-2 receptor. These observations have led some to postulate that there may be an immunological mechanism responsible for many of the symptoms seen in Persian Gulf Veterans.

However, at this time, there is little compelling evidence that any of these are major factors in the majority of symptomatic Persian Gulf Veterans. Indeed, a Department of Defense task force chaired by Dr. Joshua Lederberg was unable to pinpoint a specific cause for the symptoms seen in Gulf War veterans, suggesting that the symptoms may be due to a conflation of illnesses. This was further supported by data from a large study of Persian Gulf Veteran's which concluded that there was not a specific Persian Gulf syndrome. Moreover, at this point it is not even clear that veterans from the Gulf War have different symptoms than patients from other conflicts or for that matter civilians without military service. Nonetheless, a number of veterans returning from the Gulf are symptomatic and some do not have defined illnesses. Clearly, it is important to define the spectrum of their illnesses and determine factors that may have contributed to the development of their symptoms. Furthermore, an adequately controlled study investigating veterans who have otherwise an undiagnosed illness, to determine if there are fundamental neuropsychiatric or immunologic abnormalities would be important in either validating claims or allaying fears regarding postulates of abnormalities in these areas.

The goals of this proposal are to:

i. Evaluate self-reported conditions in Persian Gulf Veterans to determine if they represent described and accepted medical conditions and/or are associated with objective findings which would validate these symptoms.

ii. Evaluate symptomatic Gulf War Veterans to determine risk factors which may identify veterans who have reported symptoms.

iii. Evaluate in a controlled fashion symptomatic, but undiagnosed Persian Gulf War Veterans to determine if there is a definable immunologic abnormality or evidence of mycoplasma infection.

6. BODY:

Experimental Methods: To accomplish the goals of this proposal, we are performing a case control study to study the patients who presented to the Birmingham VA Hospital for evaluation of symptoms alleged to be related to the Persian Gulf Conflict. As noted above, nearly 1400 Persian Gulf veterans have been evaluated in a variety of primary and sub-specialty clinics at the Birmingham VA Medical Center, and approximately 85% of these veterans are symptomatic. These veterans will represent the symptomatic cases to be evaluated. We have established two control groups of equal numbers. The first will be randomly selected veterans of the Persian Gulf War. These individuals will be randomly selected from a complete list of veterans who have been mobilized from areas which are considered the catchment area of the BVAMC. The second control group will be composed of veterans, who were mobilized from the same areas, who have disability claims made at the same time as the cases presented for evaluation, but who were not in the Persian Gulf War. This will represent an important disability control group.

Specific Aim i: The first specific aim is to evaluate self-reported conditions in Persian Gulf Veterans and to determine if they represent described and accepted medical conditions and/or are associated with objective findings which would validate these symptoms. This group will be composed of all veterans who have presented to the BVAMC for evaluation of potential Persian Gulf related illnesses. This will be accomplished initially by chart review. Patient's complaints will be tabulated and results of evaluations and clinical impressions recorded. Diagnosis will be considered confirmed if the diagnostic impression noted by the evaluating physician fulfills standard diagnostic criteria. For example, many rheumatologic illnesses have established diagnostic criteria which have been published in the Primer on the Rheumatic Diseases. For disorders without established criteria, charts will be reviewed by two physicians and the diagnostic impression will be considered confirmed if both physicians independently concur with or arrive at the same diagnosis. If there is disagreement regarding the diagnosis, the chart will be reviewed by a third physician with expertise in the disorder being considered. Our initial analysis of the 1400 Persian Gulf veterans indicates that approximately 85% are symptomatic. Of these, approximately 10% (of the 85%) have no diagnosable illness.

In an attempt to make certain that symptoms did not predate involvement in the Persian Gulf conflict, service records will be reviewed to determine if similar complaints were present prior to individuals entering the Persian Gulf.

At the conclusion of each evaluation, patients will be placed in one of the three following categories: i. patients with symptoms and confirmed diagnosis; ii. patients with symptoms and objective examination findings, but who do not have a confirmed diagnosis and iii. those with symptoms alone and no objective findings nor diagnosis. During this review, it will also be determined if the symptomatic veterans are employed or not and if they have received formal disability. Moreover, it will be further determined if veterans have returned to formal employment or have received formal disability and the timing of each with regards to the Persian Gulf War.

As of the date of initiation of this study there were 1,541 Persian Gulf War Veterans who had been enrolled in the Birmingham registry. Careful chart reviews have been completed on 790 of them. Second physician review has been completed on 718 of these. Forty-eight individuals have an undefined illness with symptoms related to at least three organ systems. There are 72 more patients who have such symptoms, but are still scheduled to have second physician review performed. We anticipate beginning to call patients to the BVAMC early in 1998 to continue this evaluation.

Second, we have received and have categorized the list of veterans who served in the Persian Gulf from the Birmingham catchement area. This is the group of individuals who will represent our in-theater, asymptomatic individuals.

Third, we are in the process of identifying individuals in the Birmingham catchement area who were not in theater, but who have applied for disability. These individuals represent the second control group.

Specific Aim ii. To determine potential risk factors associated with Persian Gulf Veteran's becoming symptomatic. This specific aim will seek to determine if there are factors, which might have predisposed veterans to seek, care after the conflict. The symptomatic veteran group, as described in specific aim 1 will be compared to two control groups.

Control groups will be selected using Department of Defense military personal records with the assistance of Dr. Han K. Kang, Director of the Environmental Epidemiology Service VACO. The first control group will include asymptomatic individuals who were mobilized to the Persian Gulf during the same period of time. This group will control for the mobilization experience. The control individuals will be randomly selected and only matched with the symptomatic group based upon the region of the United States from where they were initially mobilized, i.e. the BVAMC catchment area. Any patient who is selected from this group who is symptomatic will be excluded. The second control group will be similarly matched, but will be drawn from individuals who were not in theater, but were activated at a similar time, are in the same catchment area, and who have disability claims. This will represent a rigorous control for disability. Each symptomatic Persian Gulf veteran will have one patient in each control group.

An analysis will be performed comparing the symptomatic veterans with the out of theater disability controls. This analysis may indicate whether there are risk factors which are specific for the Persian Gulf involvement. Since some of the symptomatic veterans are still employed or not considered disabled, it will

be important to do a sub-set analysis comparing the disabled Persian Gulf veterans to those who are not, as well as to the disabled control group.

In each group service records and, if present, prior medical records will be reviewed. If the information is not complete, individuals will be contacted by telephone. The following information will be obtained: a. specific demographic characteristics (i.e. age, race, sex, occupation, education, family income); b. service related considerations (i.e. rank, reserve vs. active duty, type of unit, years of service, performance rating (if available), and if the information is available, specific involvement in the conflict (i.e.front lines, support etc.); c. family medical history (of immediate family members to determine if there are comparable medical problems as in the symptomatic group); d. pre-existing medical illnesses; e. medications; f. smoking, alcohol or drug use history; g. vaccination history, and h. height/weight ratio at the time of mobilization. Questionnaires are being developed to capture the demographic information noted above.

Specific Aim iii: Evaluate in a controlled fashion symptomatic, but undiagnosed Persian Gulf War Veterans to determine if there is a definable immunologic abnormality or evidence of mycoplasma infection.

This aim will determine at a basic level if there are differences in a broad range of immunological parameters between symptomatic veterans and the two control groups. Identification of an abnormality based on these studies should shed light on the basic events leading to symptoms, providing insights into not only etiology, but also prognosis and treatment. This evaluation will include functional measures of specific immunity. Dr. Everson is a recognized expert in the field of primary and secondary immunity and evaluation of cytokines and related products released during the inflammatory process. His will determine if there are functional immunologic abnormalities, which might account for the symptoms seen in the Gulf War veterans. Secondly, using state of the art technology, the issue of mycoplasma infection as an etiologic agent, will be considered in symptomatic, but undiagnosed Persian Gulf veterans. Interestingly, *Mycoplasma fermentans* has been the subject of yet poorly substantiated reports attempting to link this organism with symptomatic Persian Gulf veterans. The carefully controlled and performed study described herein will use the expertise of the only state licensed Clinical Diagnostic and Reference Laboratory in the country that is solely dedicated to mycoplasma diagnosis. The methods to be used include: i) PCR detection using both mycoplasma specific primers and *M. fermentans* specific primers, ii) multiple mycoplasma culture techniques and, iii) mycoplasma specific enzyme-linked immunoassays to determine the presence of mycoplasma-specific antibodies. These studies will definitely determine if there are differences in detection of mycoplasma in the symptomatic Persian Gulf veterans as compared to controls.

Dr. Everson has established the methodology in his laboratory to determine if there are immunological differences in symptomatic Persian Gulf War Veterans and the two control groups.

Dr. Watson has continued to refine the techniques to determine if there has been evidence of mycoplasma exposure in the test and control groups. This has resulted in a manuscript, "Genotyping of *Mycoplasma fermentans* strains based on insertion sites of a mobile genetic element" that has been submitted for publication.

7. CONCLUSIONS: We are presently collecting data. Conclusions will be drawn after data collection is completed and it has been analyzed. This is anticipated at the end of year three.

8. REFERENCES: None

9. APPENDICES: None